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Research Article

IMMUNOLOGICAL AND MOLECULAR TOOLS FOR THE DIAGNOSIS IN PERIODONTICS

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ABSTRACT

Periodontitis is a common immune-inflammatory oral disease. Early detection plays an important role in its prevention and progression. Saliva is a reliable medium that mirrors periodontal health and is easily obtainable for identifying periodontal biomarkers in point-of-care diagnostics. Advanced molecular and immunological diagnostic techniques are diagnostic tests utilized to detect antigen-antibody interactions and quantify nucleic acids, proteins, or metabolites in clinical samples, facilitating the identification of risk factors, screening of asymptomatic patients, enhancing diagnostic accuracy, and informing the development of optimal therapeutic interventions. The molecular and immunological diagnostic methods that might be applied to the diagnosis and treatment of periodontitis are reviewed in this paper.

Keywords: Diagnosis, Investigation, Immunology, Periodontal tissues, Saliva.

INTRODUCTION

The periodontal tissues, which support the teeth, are impacted by the chronic, complex inflammatory disease known as periodontitis. It affects the alveolar bone, causes irreversible loss of the periodontal ligament, and, if not treated, can lead to tooth loss. An imbalance in the periodontal microbiota triggers an inadequately regulated host immunoinflammatory response, which is the primary cause of the tissue damage associated with periodontitis. Genetic factors, epigenetic modifications, systemic conditions (like diabetes mellitus), and environmental factors (like smoking, eating habits, and stress) all have a bearing. Gingival recession, tooth movement, gingival bleeding, the development of periodontal pockets, and halitosis are the clinical manifestations of periodontitis (Nazir, G., and Amin, J. 2021).

Need for investigation

Public health programs aim to raise awareness of periodontal illness so that individuals may identify its symptoms independently and seek dental treatment. Professional periodontal screening on a regular basis allows for people into three groups: dental hygiene, gingivitis, and periodontitis. Conduct a thorough periodontal evaluation and diagnosis in order to plan a comprehensive periodontitis treatment plan (Nazir, G., and Amin, J. 2021).

Classification

Laboratory investigation categories: Molecular Biology tests, PCR (Polymerase Chain Reaction), RT-PCR (Reverse Transcription PCR), DNA sequencing, Microarray analysis. Biochemical Tests, Blood glucose, Liver function tests (ALT, AST), Renal function tests (urea, creatinine), Electrolytes (sodium, potassium). Haematological Tests, Complete Blood Count (CBC), Erythrocyte Sedimentation Rate (ESR), Peripheral blood smear, Coagulation profile (PT, aPTT). Microbiological Tests, Blood culture, Urine culture, Sputum Gram stain, Acid-Fast Bacilli (AFB) staining. Immunological/Serological Tests, ELISA (HIV, Hepatitis B), Rapid antigen/antibody tests, C-Reactive Protein (CRP), Rheumatoid Factor (RF). Cytogenetic Tests, Karyotyping, Fluorescence *In Situ* Hybridization (FISH), Comparative Genomic Hybridization (CGH). Histopathological Tests, Tissue biopsy (e.g. liver, breast), Haematoxylin and Eosin (H&E), staining,

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Immunohistochemistry (IHC). Toxicological Tests, Blood alcohol level, Urine drug screen, Heavy metals (lead, arsenic) testing. Clinical Urine and Stool Analysis, Routine urinalysis, Urine microscopy, Stool for ova and parasites, Stool occult blood test. Radiological/Imaging-linked Tests, PET scan (e.g. for cancer staging), SPECT scan (e.g. for cardiac perfusion), Nuclear medicine thyroid scan (Salvi, G. E *et al*.,2023).

Immunological test

The non-covalent interactions between a viral antigen (Ag) and host antibody (Ab) form the basis of immunological tests, also known as immunoassays, which are quick to be referred to as rapid diagnostic tests (RDTs). Ag-RDTs and Ab-RDTs can be created as POC devices as well as laboratory-based diagnostic tools. Immunological diagnostic methods, as opposed to PCR, may detect earlier infections, particularly in asymptomatic people (Zhang, D *et al*.,2023).

Immunofluorescence assays

Immunofluorescent - labelled antibodies, immunofluorescence is a potent method for detecting certain target antigens. It is widely used in clinical labs as well as scientific research (Odell, I. D., and Cook, 2013). Immunofluorescence is a clinical technique that diagnoses certain skin conditions by detecting autoantibody antigen complexes under a microscope. Techniques including salt-split skin, direct immunofluorescence, and indirect immunofluorescence are employed according to the medical situation. Fluorophore-labelled antibodies that bind directly to the pathogenic auto antibody antigen complexes in the skin are used to perform direct immunofluorescence on patients' skin. The detection of circulating pathogenic autoantibodies is the primary use of indirect immunofluorescence techniques in dermatology (Odell, I. D., & Cook, 2013).

Limitations

The concentration and quality of the antibody, the specimen's processing and fluorescence signals are influenced by the employment of appropriate secondary antibodies (Odell, I. D., & Cook, 2013).

Radio-immunoassay (RIA)

An RIA requires a sample that contains the target antigen, a complementary antibody, and a radiolabelled antigen. In order to enable the sample antigen to interact with the antibody, the two are incubated together the radiolabelled antigen is added. The extracted antigen separates from the antibody due to competition with the radioactively marked antigen. The radioactive antigen's ability to bind to the antibody decreases with the amount of sample antigen present. The primary antibody can then flocculate and separate from the solution when a second antibody is introduced that binds to the first antibody. Centrifuging this mixture yields a pellet containing the bound sample protein/Radio-labelled antigen since the solution carrying the antigen–antibody complex is thicker than the one containing the free antigen. The radioactivity of the sample may be used to determine the amount of Radio-labelled antigen that has bound to the antibody and, hence, the antigen concentration in the sample (Grange, R. D *et al*.,2014).

The drawbacks

Short lifespan and the usage of a radiolabel are related to RIA. Since enzymes are not used in these experiments, there is less chance of sample-related interference (Grange, R. D *et al*.,2014)

Enzyme Linked Immunosorbent Assay (ELISA)

Some types of ELISA methods

Direct ELISA

Of all the ELISA methods, the most basic and straightforward. The microplate is first filled with the sample, then it is then incubated. The target antigen will be present in the sample. The antigen is adsorbed onto the well's surface. Only the absorbed antigen remains after the wells have been completely cleaned. After then, the well's remaining binding sites are blocked. After that, an antibody that is complementary to the target antigen is introduced to the wells, where it attaches itself to the antigen. The well is cleaned once more. As a result, a bound antigen–antibody combination remains on the well's surface. An enzyme will be affixed to the bound antibody. After that, a substrate is introduced, which the enzyme will transform into a product that can be detected. The basis for detection might be luminescence, fluorescence, or colour. With only one antibody and fewer stages than the other ELISA techniques, this approach has the benefit of being faster and easier. But it does have certain drawbacks. Many proteins that are not the target antigen will be adsorbed onto the well in complicated samples that comprise a number of different proteins. When the antigen of interest is scarce, this becomes troublesome since the test's sensitivity is decreased. The requirement that the antibody have an enzyme linked to it presents another problem. The alternative approaches do not have to repeat this expensive and time-consuming procedure for every ELISA. Additionally, the antibody's affinity for the antigen may be decreased by conjugating it with an enzyme, which would again lower sensitivity (Grange, R. D *et al*.,2014).

Indirect ELISA

After the sample carrying the desired antigen has been adsorbed onto the microplate's wells, the remaining spots on the well are blocked. After that, a complementary antibody (primary antibody) is added, and it forms a complex with the antigen. This approach is different from the direct technique in that no enzyme or other signal-generating material is coupled to the antibody when it binds to the antigen. Instead, this antibody acts as a link between the antigen and an enzyme-coupled secondary antibody. Targeting the initial antibody's Fc region, this secondary

antibody was produced by an animal different from the one that produced the original antibody. The secondary antibody will react differentially to the epitopes of the original antibody and is often polyclonal (originating from separate B cells). This enhances the test's signal and sensitivity by enabling many secondary antibodies to attach to the same main antibody., even if complex samples still have the issue of many proteins being adsorbed onto the well's surface. One benefit in this approach is that it avoids the issues mentioned above by eliminating the requirement to conjugate the main antibody. As a result, secondary antibodies may be made commercially accessible with a range of signal-producing conjugates and at a significantly reduced cost for instance, the same anti-rabbit IgG secondary antibody may be used in all ELISAs that use a primary antibody produced from rabbits (Grange, R. D *et al*.,2014).

Sandwich ELISA

Since different proteins adsorb to the well in complex samples, the sensitivity of the experiment is decreased. This is a problem for both direct and indirect approaches. This is overcome by the sandwich approach. The plate where the antigen is adsorbed to the well is first treated with a complementary antibody (capture antibody). After adding a blocking agent like previously, a sample is added. Because it can attach to the antibody, only the target antigen can stay on the plate. Now, the remainder of the experiment may be carried out similarly to a direct or indirect ELISA. Improved sensitivity is another advancement of this technique. But there is a price for it. Two antigen-specific antibodies are needed for this technique to function. They must attach to distinct epitopes on the antigen, and these must be sufficiently separated from one another to not interfere with one another's ability to bind. The capture and primary antibodies must be produced in different species if secondary antibody is being employed, as indirect ELISA. It's possible that the species that the parent antibody targets will be the target of the secondary antibody. The secondary antibody would attach to both if the primary antibody and the capture were from the same species, making the variations in the bound antigen invisible. (Grange, R. D *et al*.,2014)

Competitive ELISA

Two ligands must contend with one another for a restricted number of antibody sites in order to use this technique. One receptor will be the target antigen, and another will be a molecule that is exactly like the antigen but contains a mutation, another molecule to bind to it. One typical method is to add biotin to the target antigen. On the antibody, the biotinylated antigen and the antigen will vie for the same location. The quantity of antigen in the sample will have an inverse relationship with the signal produced by this method of testing (Grange, R. D *et al*.,2014).

Flow cytometry

The method of flow cytometry provides a rapid multi-parametric analysis of individual cells in solution. As light sources, lasers produce scattered and fluorescent light signals in flow cytometers, which are then picked up by components like photomultiplier tubes and photodiodes. A computer analyses the impulses, transforms them into electrical signals, and stores the results in a data file with a specific format (.fcs). Evaluation and/or purification of cell populations can be done using their fluorescence or light-scattering characteristics. The fluorescent reagents used in flow cytometry are diverse. These include fluorescently coated antibodies, ion indicator dyes, fluorescent expression proteins, viability dyes, and DNA binding dyes. (McKinnon, K. M. 2018)

Latex agglutination assays

Equal amounts of coloured, stabilized antigen-coated test and control latex beads are placed on an agglutination card are combined with serum to conduct the experiment. It takes two minutes to read the findings (Smits, H. L, *et al* 2000). Since, polymer colloids are employed as carriers for either antigens or antibodies, the latex agglutination test, in theory, refers to agglutination reactions between antigen and antibody via coating on polystyrene beads. LAT could be the best option because it doesn't require costly equipment, doesn't rely on a cold chain for storage or shipping, and takes little skill to conduct and read (Smits, H. L, *et al* 2000). Directness ease of use, and speed are these benefits; False negative findings and non-specific agglutination of some serum samples using uncoated latex beads are the drawbacks.

Immunoblotting (Western Blotting)

One of the most commonly used methods in Immunoblotting, also known as western blotting, is used in molecular biology labs all over the globe today. This analytical method can be used to identify and partially quantify target proteins. Additionally, by identifying certain amino acids, Western blotting may be used to detect post-translationally altered proteins that have been altered in the cell as a result of physiological alterations in both diseased and healthy conditions. Phosphorylation, ubiquitination, biotinylating, glycosylation, methylation, acetylation, sumoylation, nitration, oxidation/reduction, and nitrosylation are other post-translational modifications (PTM) that proteins may undergo (Sule R *et al*.,2021). The technique: (a)separates biological proteins from a complicated combination of internal and exterior proteins is the foundation of the idea; (b) quantifies the quantity of proteins after electrophoresing them in a gel matrix; (c) switching onto a protein-affinity membrane method; (d) "blocking" the surface of the membrane to reduce unspecific binding; (e) using antibodies that are specific to the proteins of interest to detect antigens; (f) incubating with secondary antibodies linked to a label (such as fluorescent or chemiluminescent); (g) generating and identifying a signal that is essentially related to the level of

antibody/antigen interaction; and (h) measuring the bands that are produced (Bass, J.J *et al* 2021).

Polymerase Chain Reaction (PCR)-based methods

A novel and well-liked molecular biology method for enzymatically reproducing DNA without the need of a live creature, such yeast or E. coli, is the polymerase chain reaction (PCR). The method makes it possible to exponentially multiply a little quantity of the DNA molecule many times (Rahman M. T. *et al* 2013). The four main ingredients are needed for PCR: nucleotide triphosphates, thermostable DNA polymerase. (Ishmael, F. T, and Stellato C.,2008).

Loop-Mediated Isothermal Amplification (LAMP) technique

Researchers from a wide range of disciplines are still interested in the well-established technique known as loop-mediated isothermal amplification (LAMP) (Mori, Y *et al*.,2013). In recent years, there have been two main areas of concentration for LAMP technology research and development. One is its usefulness in clinical settings, where it may be used to enhance current tests. Since the invention of the LAMP technique, other research groups have created LAMP reactions for the detection of different infections, and their performance has been evaluated by comparing it to that of currently available reagents (PCR). Following these thorough validation trials, LAMP was used in clinical settings. Basic research on further simplification of the LAMP test is the second topic. To incorporate this technique into straightforward genetic testing for use as point-of-care diagnostics, significant advancements in LAMP technology are required (Mori, Y *et al*.,2013).

DNA-DNA checkerboard hybridization methods

Techniques for DNA–DNA hybridization (DDH), sometimes referred to as DNA–DNA reassociation techniques, are predicated on an effort to compute the total genomic similarity between various species by doing raw comparisons of their whole genomes (Rosselló-Móra *et al*.,2011). One technique that offers more resolution than 16S rDNA sequencing and the 70% requirement is DNA-DNA hybridization (Cho, J. C., and Tiedje, J. M. 2001). This technique makes it possible to identify several microorganisms in a large number of samples at once by identifying gram-positive and gram-negative bacterial species present in subgingival biofilms using up to 45 complete genomic DNA probes (do Nascimento, C *et al*.,2009).

Fluorescence in situ hybridization (FISH)

The FISH technique is helpful for many applications in all areas of microbiology and enables the simultaneous imaging, identification, enumeration, and localization of individual bacterial cells. Because FISH allows for the discovery of both culturable bacteria and unculturable species that have not yet been produced, it can help with the understanding of complex microbial ecosystems (Moter, A., and Göbel, U. B. 2000). Nucleic acids in their biological surroundings can be spatially detected and quantified using this method. It has become a potent cytogenetic technique for transcriptome study of cells and tissues. The FISH signal is detected using two different combinatorial labelling techniques, spectral karyotyping and M-FISH, which are based on the presence or absence of certain fluorophores or on the spectrum characteristics. The full set of 24 human chromosomes may be specifically labelled using both techniques, which can identify translocations, insertions, deletions, and other abnormalities (Moter, A., & Göbel, U. B. 2000). A method that can help with prognostic outcome prediction as well as genetic disease diagnosis, FISH can identify genetic anomalies such distinct gene fusions, aneuploidy, chromosomal loss or partial chromosome loss, or the progression of an aberration. (Bishop, R. 2010).

Microarray technology

Although they are also used to analyse protein and small molecule libraries, microarrays are now the primary technique for evaluating gene expression through cDNA or RNA analysis. Microarrays have made it possible to use inexpensive, high-throughput tests in the drug development process and have assisted in extracting more information from fewer sample quantities (Howbrook DN *et al* 2003).

Sequencing methods

By using this technique, chain-terminating and radioactively (Hu, T., Chitnis *et al*., 2021). Novel and quick methods mRNAs, short RNAs, transcription factor regions, chromatin structure, DNA methylation patterns, microbiology, and metagenomics are all characterized and profiled genome-wide using next-generation sequencing technology. (Ansorge, W. J. 2009)

Mass spectrometery

One common experimental technique for separating electrically charged substances in a gaseous state is mass spectrometry (MS). The charged species, or ions, are produced by the ion source. Sometimes the ion source makes it easier for liquid or solid analytes to enter the gas phase. The gas-phase ions are then transferred to the mass analyser. The mass analyser categorizes the ions either geographically or chronologically based on the mass-to-charge ratios (m/z). The separated ions are found using an ion detector in the time or space domain. Electric signals produced by the ion detector are processed to produce mass spectra (Urban, P. L. 2016).

Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance spectroscopy (NMR) is a spectroscopic technique used to investigate the local magnetic fields around atomic nuclei. It is also sometimes referred to as magnetic resonance spectroscopy (MRS) or NMR spectroscopy. The NMR signal, which is produced when radio waves cause nuclear magnetic resonance in the sample's nuclei while it is in a magnetic field, is picked up

by sensitive radio receivers. Modifying the resonance frequency generated by the intramolecular magnetic field surrounding an atom provides information on certain functional groups and a molecule's electrical structure. Given that some compounds have fields that are either very unique or unique, NMR spectroscopy is the gold standard in modern organic chemistry practice for identifying organic compounds that are single molecules. Similarly, proteins and other complicated compounds are identified by biochemists using NMR (Rabenstein DL., & Guo W. 1988).

CONCLUSION

Immunological and molecular diagnostic methods have greatly changed the early identification and management of periodontal diseases. These approaches provide a more nuanced understanding of disease activity and progression, as well as risk estimation for specific patients, because they allow for the determination of specific biomarkers in saliva, gingival crevicular fluid, and other clinical specimens. Improvements in anti–antibody assays, nucleic acid-based tests, and metabolomic analyses not only improve diagnostic accuracy, but also support rapid chairside assessments to enhance clinical management of periodontal patients, while the immune-inflammatory condition that defines periodontal disease can benefit from the integration of these measurements into everyday practice making it possible to detect disease before it is clinically observable, predicting disease outcomes, and personalizing treatment based on detected disease activity. The more immunological and molecular diagnostic tests develop and become validated, it is likely that their clinical relevance will continue to be validated and utilized to improve patient care and periodontal health in the long term.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

REFERENCES

Ansorge W. J. (2009). Next-generation DNA sequencing techniques. *New Biotechnology*, *25*(4), 195–203.

Bass, J. J., Wilkinson, D. J., Rankin, D., Phillips, B. E., Szewczyk, N. J., Smith, K., & Atherton, P. J. (2017). An overview of technical considerations for Western blotting applications to physiological research. *Scandinavian Journal of Medicine & Science in sports*, *27*(1), 4–25.

Bishop, R. (2010). Applications of fluorescence in situ hybridization (FISH) in detecting genetic aberrations of medical significance. *Bioscience Horizons*, *3*(1), 85-95.

Cho, J. C., & Tiedje, J. M. (2001). Bacterial species determination from DNA-DNA hybridization by using genome fragments and DNA microarrays. *Applied and Environmental Microbiology*, *67*(8), 3677-3682.

do Nascimento, C., Ferreira de Albuquerque Junior, R., Issa, J. P. M., Ito, I. Y., Lovato da Silva, C. H., de Freitas Oliveira Paranhos, H., & Freitas de Souza, R. (2009). Use of the DNA Checkerboard hybridization method for detection and quantitation of Candida species in oral microbiota. *Canadian Journal of Microbiology*, *55*(5), 622-626.

Grange, R. D., Thompson, J. P., & Lambert, D. G. (2014). Radioimmunoassay, enzyme and non-enzyme-based immunoassays. *British Journal of Anaesthesia*, *112*(2), 213-216.

Howbrook, D. N., van der Valk, A. M., O'Shaughnessy, M. C., Sarker, D. K., Baker, S. C., & Lloyd, A. W. (2003). Developments in microarray technologies. *Drug Discovery Today*, *8*(14), 642-651.

Hu, T., Chitnis, N., Monos, D., & Dinh, A. (2021). Next-generation sequencing technologies: An overview. *Human Immunology*, *82*(11), 801-811.

Ishmael, F. T., & Stellato, C. (2008). Principles and applications of polymerase chain reaction: basic science for the practicing physician. *Annals of Allergy, Asthma & Immunology*, *101*(4), 437-443.

McKinnon, K. M. (2018). Flow cytometry: an overview. *Current Protocols in Immunology*, *120*(1), 5-1.

Mori, Y., Kanda, H., & Notomi, T. (2013). Loop-mediated isothermal amplification (LAMP): recent progress in research and development. *Journal of Infection and Chemotherapy*, *19*(3), 404-411.

Moter, A., & Göbel, U. B. (2000). Fluorescence in situ hybridization (FISH) for direct visualization of

microorganisms. *Journal of Microbiological Methods*, *41*(2), 85-112.

Nazir, G., & Amin, J. (2021). Molecular tools for the diagnosis of periodontitis. *International Journal of Dental Research*, *6*(3), 81-8.

Odell, I. D., & Cook, D. (2013). Immunofluorescence techniques. *The Journal of Investigative Dermatology*, *133*(1), e4.

Rabenstein, D. L., & Guo, W. (1988). Nuclear magnetic resonance spectroscopy. *Analytical chemistry*, 60(12), 1R–28R.

Rahman, M. T., Uddin, M. S., Sultana, R., Moue, A., & Setu, M. (2013). Polymerase chain reaction (PCR): a short review. *Anwer Khan Modern Medical College Journal*, *4*(1), 30-36.

Rosselló-Móra, R., Urdiain, M., & López-López, A. (2011). DNA–DNA Hybridization. In *Methods in microbiology* (Vol. 38, pp. 325-347). Academic Press.

Salvi, G. E., Roccuzzo, A., Imber, J. C., Stähli, A., Klinge, B., & Lang, N. P. (2023). Clinical periodontal diagnosis. *Periodontology 2000*, 10.1111/prd.12487. Advance online publication. https://doi.org/10.1111/prd.12487

Smits, H. L., van der Hoorn, M. A., Goris, M. G., Gussenhoven, G. C., Yersin, C., Sasaki, D. M., ... & Hartskeerl, R. A. (2000). Simple latex agglutination assay for rapid serodiagnosis of human leptospirosis. *Journal of Clinical Microbiology*, *38*(3), 1272-1275.

Smits, H. L., van der Hoorn, M. A., Goris, M. G., Gussenhoven, G. C., Yersin, C., Sasaki, D. M., ... & Hartskeerl, R. A. (2000). Simple latex agglutination assay for rapid serodiagnosis of human leptospirosis. *Journal of Clinical Microbiology*, *38*(3), 1272-1275.

Sule, R., Rivera, G., & Gomes, A. V. (2023). Western blotting (immunoblotting): history, theory, uses, protocol and problems. *Biotechniques*, *75*(3), 99-114.

Urban, P. L. (2016). Quantitative mass spectrometry: an overview. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, *374*(2079), 20150382.

Zhang, D., Kukkar, D., & Kim, K. H. (2023). Immunological tests: Essential and supportive tools in the fight against COVID-19. *TrAC Trends in Analytical Chemistry*, *168*, 117291.